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POSSIBILITIES OF PREPARING LACTIC ACID FROM GRAPEFRUIT JUICE*

by

Arthur J. Nolte and Harry W. von Loesecke,
U. S. Citrus Products Station, Bureau of Agricultural Chemistry & Engineering,
U. S. Department of Agriculture, Winter Haven, Florida

Introduction

Because of the increasing production, and decline in price, of both fresh and canned grapefruit, the problem of developing new uses for citrus, and particularly grapefruit, has become of great importance. The production of power alcohol has been suggested by some. When it is considered that the theoretical yield of industrial ethyl alcohol (190 proof) per ton of grapefruit is only about 4.31 gallons**, the economics of the problem become questionable. Experimental work on the preparation of butyl alcohol and butyric acid from grapefruit has been carried out at this station. Yields were so low that commerical production would not be practical. Attention was therefore directed toward the feasibility of preparing lactic acid from grapefruit.

In present commercial practice, lactic acid may be produced from starch (corn or potato), molasses (cane or beet), and dextrose by fermentation with lactic acid bacteria of the Lactobacillus delbruckii type. In recent years considerable work has been accomplished in the production of lactic acid from whey[1,2]. The total lactic acid production in the United States in 1938 was said to have been five million pounds (100% acid).

Experimental

The fruit used was cull and unsuitable for the fresh fruit market or for canning. The juice was extracted by hand reaming with subsequent screening through a 20 mesh screen to remove pulp and seeds. The calculated amount of calcium carbonate (which should be free of iron and low in magnesium) was then added to neutralize the citric acid present. The use of calcium oxide or hydroxide in place of carbonate was unsatisfactory because of lumping and poor distribution. The precipitated calcium citrate was allowed to remain in the juice during the period of fermentation; no harm was caused by this procedure and it avoided an extra filtration.

Fermentation was carried out by lactobacilli normally present on the fruit. Better results were obtained by using these organisms than by utilizing pure cultures of lactic acid bacteria. The microbiology of the process is being further studied.

It was found advantageous to prepare starters, where large volumes of juice are to be fermented. This saved time and aided in acclimating the organisms to the medium. The starter was prepared by maintaining the juice (previously neutralized with calcium carbonate) at 50°C. (122°F.) for two to three days.

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** This is assuming a yield of juice of 85 gallons per ton and with a sugar content of 7.4 per cent. At the present market price of 0.0295 per gallon for industrial alcohol, this would amount to $1.27.

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The main batch of juice was warmed to 50° C. (122° F.) and the starter (about 5% by volume of the main batch) added. During fermentation periodic neutralization with calcium carbonate was required, the pH being maintained between 4.0 and 6.5. The reason for this is that the organisms are capable of producing only about 1.5% free lactic acid, at which point their activity is inhibited unless the free lactic acid is neutralized. The pH of the fermenting liquor changes because of the formation of lactic acid. Where attempts were made to maintain the pH within a narrow range by the addition of an excess of calcium carbonate, fermentation was slower than when periodic neutralization was carried out. The organisms may require some fluctuation from an optimum pH and temperature range, rather than any one fixed environmental condition[3].

Fermentation required from six to eight days for completion. Maintenance of fermentation temperatures in the range of 50° - 55° C. (122°-131° F.) was found to be of great importance. At lower temperatures, acetic, propionic and butyric acid forming organisms developed with a resulting increase in volatile acids of the mash. At higher temperatures the lactobacilli were weakened, or even destroyed.

The ratio of surface area to volume of mash was also found to be of importance. Since lactobacilli are micro-aerophilic large amounts of air were found detrimental to complete fermentation. In distilling practice exclusion of oxygen from the sour mash tubs is accomplished by the use of tubs with removable covers and a ratio of surface area to volume of about one or two square inches per gallon. In our experiments, a ratio as low as this was never attained; it ranged from 12 to 200 or more square inches per gallon. The higher surface area ratios per gallon of juice resulted in decreased yields of lactic acid, and stoppage of fermentation before completion. As an example, in an experiment where the surface area-volume ratio was 200 sq. in. to one gallon, the fermentation had ceased in six days with a sugar consumption of 54.5%. Where the surface area-volume ratio was 20 sq. in. to one gallon, the sugar consumption amounted to 87.6% in six days.

Theoretically, one molecule of dextrose is capable of yielding two molecules of lactic acid:

$$C_6H_{12}O_6 = 2C_3H_6O_3$$

or 100% of the weight of the inverted sugar. Certain species of lactic acid bacteria will convert over 98% of dextrose into inactive dl-lactic acid. Practically, sugar conversions do not amount to more than 90% of the theoretical, while 70% fermentation yields of lactic acid, based on sugar converted, are considered profitable, provided the raw material is cheap. In our work, yields of lactic acid, based on sugar converted, ranging from 71 to 84% were obtained from grapefruit juice (Table 1).

Method of Determining Lactic Acid in the Fermented Mash. A sample of the mash was neutralized with calcium oxide and filtered. A measured portion of the filtrate was heated to boiling and an excess of 0.5 N ammonium oxalate added. The mixture was boiled a short time, allowed to stand for 30 minutes and then filtered. The precipitate was washed with warm water containing a small amount of ammonium hydroxide and ammonium oxalate. The washed precipitate was ignited to constant weight over a blast lamp and the ignited precipitate weighed as calcium oxide. A measured portion of the same sample was analyzed for volatile acids according to regular methods[4]. The calcium oxide equivalent of the volatile acids (assuming these acids to be acetic) was then calculated according to the reaction:

$$CaO + 2HC_2H_3O_2 = Ca(C_2H_3O_2)_2 + H_2O$$

This equivalent was then substracted from the calcium oxide found above and the value obtained multiplied by 3.21 to obtain the lactic acid content of the fermented mash.

Recovery of Calcium Lactate from Fermented Mash. After fermentation was complete, calcium oxide or hydroxide was added until the batch was alkaline to phenolphthalein. No lumping or poor distribution of the chemical was experienced at this point because of the very small amount added. The liquor became dark at this stage. If allowed to remain acid, recovery of crystalline calcium lactate was rendered difficult.

The precipitated calcium citrate was removed by means of a plate and frame press, the cake washed with hot water and the washings added to the main batch. About 93% of the calculated amount of citric acid was recovered as calcium citrate.

The combined filtrate and washings from the calcium citrate were treated hot with activated carbon (2% based on the weight of the liquor) and pumped through a plate and frame press using infusorial earth as a filter aid. The filtered liquor, light amber in color, was evaporated at atmospheric pressure to 21° Be. Concentration in vacuo yielded a lighter colored crude lactate, but since concentration at atmospheric pressures requires less expensive equipment, this was the procedure finally adopted. Upon standing, calcium lactate crystallized from the concentrated liquor. The lactate was washed, the washings and mother liquor again being concentrated (to 25° Be) and a further crop of lactate obtained. Concentrations of the washings and mother liquor were repeated at 28°, 31° and 34° Be. Obviously, in industrial practice there would be considerably less concentration of the wash waters and mother liquor, for these would be added to the original fermented liquor. Dark, commercial lactic acid was obtained from the 34° Be filtrate by the addition of sulfuric acid and removal of the precipitated calcium sulfate.

For U.S.P. calcium lactate, the washed lactate was dissolved in hot water, treated with activated charcoal (1% based on the weight of the lactate) filtered and allowed to crystallize.

Preparation of Lactic Acid from Calcium Lactate: The damp calcium lactate was made into a thick slurry with water, taking care the mass was free of lumps. The calculated amount, plus a slight excess, of concentrated sulfuric acid was then added followed by activated carbon. The mixture was heated for about 10 minutes slightly below the boiling point, and then filtered and washed. At this point the solution was water white and contained about 10% lactic acid. Excess sulfuric acid was then removed with barium hydroxide, the solution again filtered and the cake washed.

The lactic acid, now free of sulfates and calcium, was evaporated in vacuo to 44%. At this point the acid was light amber due to the presence of iron. The iron content was determined according to the thiocyanate method used in water analysis. Knowing the amount of iron present, sufficient potassium ferrocyanide was added to partly precipitate the iron but leaving about 5 ppm in solution. If the entire amount of iron were precipitated a colloidal solution of ferric ferrocyanide was obtained coloring the acid a greenish hue which could not be removed. The iron-free 44% acid was decolorized with char and concentrated in vacuo to 50%.

Yields of Calcium Lactate and Lactic Acid from Grapefruit. It will be seen from Table I that actual recovery of U.S.P. calcium lactate averaged 6.0%, based on the weight of the juice taken. This represents a loss of 22% of the amount of lactate actually present in the fermented juice. On the basis of the experiments, a ton of grapefruit would yield 46.5 pounds of calcium lactate, assuming 85 gallons of juice to the ton with a fermentable sugar content of 7.4%.

In the case of lactic acid (50% strength), there was obtained an actual recovery of 7.1%, based on the weight of the juice taken. This represents a loss of 36% of the amount of lactic acid actually present in the fermented juice. In the case of commercial production of lactic acid (50% strength) from whey, Burton[1] cites losses of 45%.

It is quite likely greater yields of calcium lactate and lactic acid could be obtained on a commercial scale because of greater ease in controlling the temperature, and maintaining fermentation more free of oxygen.

Assuming an average citric acid content of 1.8% in mature fruit, the acid content of one ton of fruit would amount to 13 pounds. In our experiments, 93% of the citric acid was recovered as crude anhydrous calcium citrate. Thus, a ton of fruit on this basis would yield about 15.7 pounds of anhydrous calcium citrate. This material would be considered as a by-product in the manufacture of calcium lactate and lactic acid.

Summary

A method has been described for preparing calcium lactate and lactic acid from cull grapefruit juice.

Yields of lactic acid ranged from 71 to 84%, based on sugar converted. Actual recovery of calcium lactate averaged 6.0%, based on the weight of the juice; recovery of 50% lactic acid averaged 7.1%, based on the weight of the juice.

Calcium citrate, at the rate of 15 pounds per ton of fruit, is obtained as a by-product.

Literature Cited

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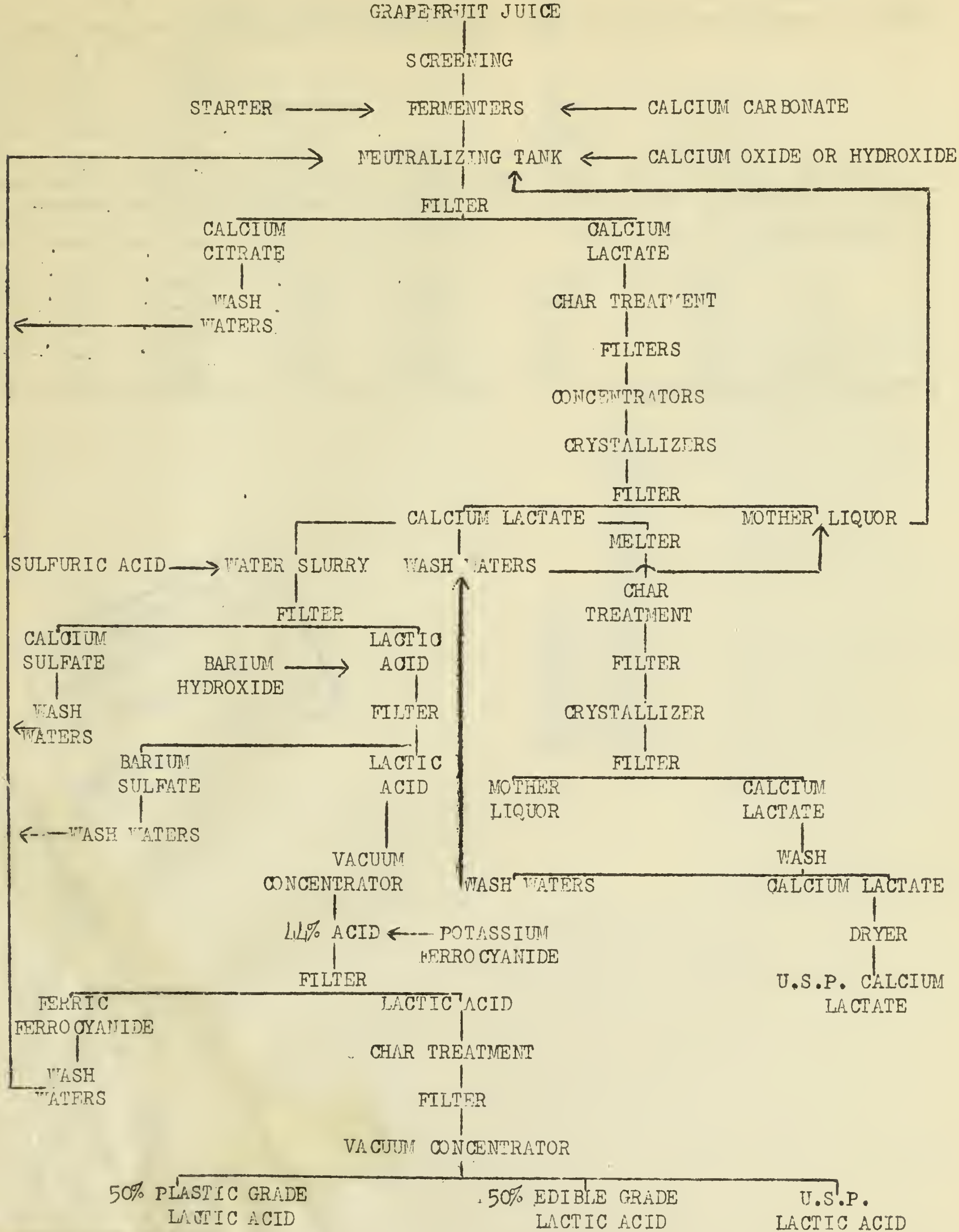

Flow Diagram for Production of Calcium Lactate and Lactic Acid from Grapefruit Juice.

Table 1 - Fermentation of Grapefruit Juice for the Production of Lactic Acid

Exp. No.	Quality of Juice taken cc.	Total Sugars as Invert before fermentation %	Total Sugars as Invert after fermentation %	Sugar Consumption %	Yield of Lactic Acid (100%) as determined by analysis %	Fermentation Yield of Lactic Acid, % of sugar Converted	% U.S.P. Cal. lactate recovered*	% 50% Lactic acid recovered*
1	200	7.19	1.38	80.8	5.35	74.4	6.0	6.5
2	200	7.19	0.40	94.4	6.05	84.1	6.1	6.7
3	7,570	7.50	1.79	76.1	5.32	70.9	5.1	...
4	15,140	7.48	0.93	87.6	5.31	71.0	6.1	...
5	75,700	7.28	0.60	91.7	5.60	76.9	5.6	8.1

* Based on weight of juice.

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